(Amended - Clean version)

It should be understood that any macromolecule capable of specifically recognizing and binding to an analyte may be a sensing element of the invention. Such macromolecules (also known in the art as bioaffinity agents) rely on molecular recognition of an analyte as the first step in the generation of a response by a biochip. Strategies for selecting the appropriate bioaffinity agent for an analyte of interest are well known in the art (See Chapter 3 in Kress-Rogers, E. 1997, *Handbook of Biosensors and Electronic Noses: Medicine, Food, and the Environment*, [ed., Kress-Rogers, E.], CRC Press, Boca Raton). A sensing element is further defined to be any macromolecule that when activated by the binding of a chemical ligand or specific analyte, in combination with the addition of appropriate optical labels, causes generation of a detectable optical signal, and operates to communicate the presence of said ligand or analyte from the sample comprising analytes or ligands for detection through an optical reader. The sensing elements of the invention are capable of detecting thousands of natural and synthetic molecular species (analytes). In principal, the range of chemical structures that can be detected by sensing elements in the device and methods of the invention is unlimited (Kress-Rogers, E. 1997, *Handbook of Biosensors and Electronic Noses: Medicine, Food, and the Environment*, [ed., Kress-Rogers, E.], CRC Press, Boca Raton). According to the conditions of the detection method, the binding of the analyte to the sensing element yields a luminescent event (e.g fluorescence, phosphorescence) that is, a signal, which,

when processed or monitored by an optical reader indicates the presence or concentration of an analyte of interest in the target sample.

(Amended - Clean version)

Luminescent Event/Fluorescence

In luminescent-event detection, and in particular fluorescence-detection methods, a fluorescent molecule has the ability to absorb photons of energy at one wavelength and subsequently emit the energy at another wavelength. Fluorescence is caused by incident radiation impinging upon or exciting an electron of a molecule. The electron absorbs the incident radiation and is raised from a lower quantum energy level to a higher one. The excess energy is released as photons of light as the electrons return to the lower, ground-state energy level. See *"Bioconjugate Techniques,"* Greg T. Hermanson, Academic Press, San Diego, CA, (1996), and *"Principles of Biochemistry,"* Lehninger, et al., 2nd ed., 1993, for a detailed description of fluorescent labeling and detection techniques.

(Amended - Clean version)

Markers/Reporter Probes

As used herein, a "marker" is any agent that can be used to label proteins, nucleic acids, and other molecules as disclosed in the context of the present invention. One of skill in the art selects appropriate markers required to suit the detection method used and needs of the assay. A "reporter probe" refers to a labeled molecule that yields a luminescent event upon exposure to excitation energies. The reporter probes of the present invention possess a selective affinity (i.e., a tendency to react or combine with atoms or compounds of different chemical constitution (*See* Hawley's Condensed Chemical Dictionary, 13th ed., Richard L.

(Amended - Clean version)

It should be understood that the terms "marker" and "reporter probe" in context may be used interchangeably. Since each marker has its own luminescent character, more than one labeled molecule, each tagged with a different marker, can be used at the same time to detect two or more analytes of interest. Markers known in the art that are useful for the present invention include, but are not limited to, Cyanine 3 (Cy3), Cyanine 5 (Cy5), and those enumerated in *"Microarray Biochip Technology,"* Mark Schena (ed.), Eaton Publishing, Natick, MA, 2000; *"Bioconjugate Techniques,"* Greg T. Hermanson, Academic Press, San Diego, CA, (1996); *"Principles of Biochemistry,"* Lehninger, et al., 2nd ed., 1993; *"Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology,"* Shan-Rong Shi, Jiang Gu, Clive R. Taylor (eds.), Eaton Publishing, Natick, MA, 2000; *"Immunological Reagents & Solutions: A Laboratory Handbook,"* Bassam B. Damaj, Eaton Publishing, Natick, MA, 2000; *"Protein Staining and Identification Techniques,"* Robert C. Allen, and Bruce Budowle, Eaton Publishing, Natick, MA, 1999; *"Affinity and Immunoaffinity Purification Techniques,"* Terry M. Phillips, and Benjamin F. Dickens, Eaton Publishing, Natick, MA, 2000.

(Amended - Clean version)

Although agents which cross-link vis-a-vis covalent bonds are widely used, one of the most popular methods of noncovalent conjugation is to make use of the natural strong binding of avidin for the small molecule biotin. A preferred cross-linking agent involves avidin. Without limitation, avidin can be strepavidin, modifications, thereof, or avidin. Avidin can be coupled with agarose by various chemistries. Ideally, strepavidin is embedded in the surface layer of the matrix coating, binding essentially irreversibly to biotinylated sensing elements (e.g. sensing elements which are nucleic acids). See *"Bioconjugate Techniques,"* Greg T. Hermanson, Academic Press, San Diego, CA, (1996). Strepavidin (or any protein containing a lysine) will react with the aldehyde of Nufix™, a commercially available aldehyde-activate matrix. Cross-linking system for linking a proteinacious capture probe to the platform of the invention are well known, *ibid.*

(Amended - Clean version)

In a preferred embodiment of the invention, the reporter probes (75) contain either the Cy3 or Cy5 marker (65) and are deposited onto the top surface (6) of the matrix (5) following adhesion of the analyte of interest (85) to the sensing element (25). The reporter probe (75) will react, combine, or otherwise bind to an analyte (85) of interest, thereby causing a luminescent effect upon exposure to excitation energy. This luminescent effect indicates the presence of the analyte of interest (85). Reporter probes that are design-engineered specifically to the user's requirements are commercially available from NEN Life Science Products, Boston, MA.

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Experiment 8 - Labeled Sensing Element Cross-Linked to a Platform

This experiment was performed to show that a fluorescently-labeled sensing element produces a detectable luminescent effect (i.e, fluorescence) when bound to the top surface of a platform. The procedure of experiment 7 was repeated. Then an aliquot of Oligo A from experiment 4 was deposited over the surface of the matrix and allowed to cross-link. Using the confocal microscope and laser of experiment 1, an image was detected over every 200 micron area of the surface of the matrix.

Experiment 9 - Unlabeled Sensing Element Produced No Image

This experiment was performed to show that an unlabeled sensing element bound to the surface of the matrix does not produce a luminescent effect. The procedure of experiment 7 was followed using Oligo B (NEN Life Science Products). Using the confocal microscope and laser of experiment 1, no image was detected.

Experiment 10 - Detecting a Reporter Probe Bound to a Sensing Element

This experiment was performed to show that a fluorescently-labeled analyte (e.g., nucleic acid) that hybridizes to a sensing element bound to the surface of the matrix produces a luminescent effect. The procedure of experiment 9 was repeated. An aliquot of Oligo C (NEN Life Science Products), which is partially complementary to Oligo A, was then

deposited over the surface of the matrix and allowed to hybridize with Oligo B. This experiment was performed in triplicate, allowing Oligo C to hybridize for 4 hours, 8 hours, and overnight. Using the confocal microscope and laser of experiment 1, images were detected over every 200 micron area of the surfaces of each of the matrices.

(Amended - Clean version)

Experiment 11 - Formation of a Biochip

This experiment was performed to show that unlabeled nucleic acid sensing elements spotted onto the surface of the matrix do not produce a luminescent effect. A 210 micron pin was obtained from TeleChem International, Inc., Sunnyvale, California. A corresponding 4-spot metal spotting block was machined at a local metal fabricating shop such that the weight of the pin spotter determines the amount of oligo that is deposited. The procedure of experiment 7 was repeated. Two spots of Oligo B from experiment 9 were then spotted onto the surface of the matrix. Using the confocal microscope and laser of experiment 1, no image was detected. The device was then washed with deionized water and placed in the confocal microscope for another reading. No image was detected.